When Things Go Wrong

Contingency & Adjustment Protocol — V2

Companion to the 3R Senolytic Study Protocol V2

Updated for 3-arm dose-finding + tissue biomarkers

Adamia Research — August 2026

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Introduction

For each measurement in the V2 study, we imagine unexpected results and plan adjustments. With the 3-arm design (placebo / 500mg / 1,000mg), some scenarios from V1 are now resolved by design. New scenarios emerge from the tissue biomarkers.

Scenario 1: Tissue p16 Doesn't Decrease (Both Doses)

> ⚠️ **What If This Happens?**
>
> **Problem**: IHC on skin biopsies shows no reduction in p16+ fibroblasts at Day 120 in either treatment arm vs. placebo.

Possible Reasons

1. **Even 1,000mg is sub-therapeutic in tissue**: Skin fibroblasts are in deep dermis with low blood flow. Orally-delivered fisetin may not reach therapeutic concentrations in dermal tissue.
2. **16 weeks is too short for tissue clearance**: Killing fibroblasts is one thing; replacing them with new ones takes months. The biopsy at Day 120 may still show debris from recently-killed cells.
3. **Fisetin + piperine interaction**: Piperine inhibits liver enzymes (CYP3A4) but may also accelerate fisetin metabolism through alternative pathways.

> 🔧 **Adjustment**
>
> ### Adjustments
>
> - **Add topical fisetin arm in V3**: Apply fisetin cream directly to the biopsy site region. Compare oral vs. topical vs. combined. This bypasses bioavailability entirely.
> - **Extend to 9 months**: A 9-month extension on the same participants (open-label, all receiving 1,000mg) with biopsies at Month 6 and Month 9.
> - **Measure fisetin in tissue**: Use LC-MS/MS on the biopsy tissue itself to confirm fisetin reached the fibroblasts. If not detectable → bioavailability problem. If detectable but p16 unchanged → fisetin doesn't kill human fibroblasts at achievable concentrations.
> - **Switch to dasatinib + quercetin**: If fisetin definitively fails, D+Q is the alternative senolytic with human clinical data (Hickson 2019).

Scenario 2: Dose-Response Without Significance

V2

⚠️ **What If This Happens?**

Problem: p16 decreases by 5% (placebo), 12% (500mg), 25% (1,000mg) — a clear dose-response trend — but none reaches $p < 0.05$ individually.

What This Means

This is actually a **strong positive signal**. The linear trend test (Cochran-Armitage) can detect this even when individual comparisons fail. With n=15, we expect to be underpowered for individual arms but powered for trends.

🔧 **Adjustment**

- **Report the trend**: A significant linear trend ($p < 0.05$) with dose-response is publishable and fundable. It says "it's working, we just need more people."
- **Power calculation for V3**: Use the observed effect size to calculate the sample needed for a definitive study. If the 1,000mg arm shows d=0.6, V3 needs ~45 people per arm.
- **Do NOT increase dose further**: 1,000mg + piperine is already ambitious. The answer is more participants, not more fisetin.

Scenario 3: IL-6 Increases (Both Doses)

> ⚠️ **What If This Happens?**
>
> **Problem**: IL-6/IL-8 spike in both treatment arms at Day 30, persisting through Day 120.

Reasons

1. **Clearance spike**: Senescent cells dying → dump SASP contents → transient inflammation.
2. **1,000mg dose is hepatotoxic**: Liver irritation from high-dose fisetin + piperine.

> 🔧 **Adjustment**
>
> - **Check liver panel immediately**: If ALT/AST normal → likely clearance spike. If elevated → hepatotoxicity, stop high-dose arm.
> - **Time-course sub-study**: Draw blood at Day 2, 4, 7 after first pulse to map the inflammatory spike precisely.
> - **Add CRP and TNF-α**: Broaden the inflammatory panel. If CRP is normal despite high IL-6, the inflammation is localized (tissue clearance), not systemic.
> - **If clearance spike is confirmed**: This is expected and good. Document the kinetics. In V3, add an anti-inflammatory bridge (curcumin 500mg or omega-3 2g on non-pulse days).

Scenario 4: NAD+ Unchanged

⚠️ **What If This Happens?**

Problem: Blood NAD+ doesn't increase despite 250mg NMN daily.

🔧 **Adjustment**

- **Increase to 500mg/day** or switch to sublingual NMN (bypasses GI degradation).
- **Switch to NR (Nicotinamide Riboside)**: More stable, better studied in humans.
- **Add apigenin (50mg) or quercetin (500mg)**: Natural CD38 inhibitors. Without blocking CD38, NAD+ may be degraded as fast as it's produced.
- **Verify NMN purity**: Third-party CoA required. Some "NMN" supplements are mostly nicotinamide.
- **Measure intracellular NAD+**: Switch from plasma to whole-blood lysate assay.

Scenario 5: Tissue SDF1 Doesn't Increase

> ⚠️ **What If This Happens?**
>
> **Problem**: SDF1 in skin biopsy unchanged despite reduction in p16 (senescent cells were killed).

Reasons

1. **SDF1 recovery requires new fibroblasts**: Killing senescent fibroblasts removes the cells that produce low SDF1, but new fibroblasts must migrate and proliferate to restore normal SDF1. This takes months.
2. **SDF1 regulation is independent of senescence**: Maybe SDF1 is controlled by UV exposure, hormones, or other factors we're not addressing.

> 🔧 **Adjustment**
>
> - **Extend biopsy timeline**: If p16 decreased but SDF1 didn't, biopsy again at Month 6 or 9 to give new fibroblasts time to establish.
> - **Accept SDF1 as exploratory**: If p16 and SA-β-gal improve, the treatment is working regardless of SDF1. SDF1 may be a downstream consequence rather than a driver.

Scenario 6: Skin Spots Don't Improve (Despite Biomarker Improvement)

⚠️ **What If This Happens?**

Problem: Tissue p16 decreased, IL-6 decreased, NAD+ increased — but brown spots look the same in photos.

Reasons

1. **Dermal melanin is very slow to clear**: The epidermis renews every 28 days, but dermal melanin (where deep solar lentigines live) can persist for years.
2. **Melanin quantification (Masson-Fontana) may show improvement invisible to photography**: A 20% reduction in melanin content may not be visible but is measurable.

🔧 **Adjustment**

- **Trust the tissue data over photos**: If Masson-Fontana shows reduced melanin, that's more reliable than visible assessment. Report it.
- **Extend to 9-12 months for visible results**: The biomarkers lead; visible changes follow by months.
- **Combine with dermatological procedure**: Microneedling (as in Yoon's study) may be needed to physically remodel the pigmented tissue after senescent cells are cleared.

Scenario 7: High-Dose Arm Shows Liver Toxicity

V2

⚠️ **What If This Happens?**

Problem: 2+ participants in the 1,000mg arm develop ALT >3× ULN at Day 30.

🔧 **Adjustment**

- **Stop the 1,000mg arm immediately** (per pre-specified rule). Safety Monitor reviews all data.
- **Continue the 500mg and placebo arms** (unless liver issues there too).
- **Reduce high dose to 750mg in V3** — the window between therapeutic and toxic may be narrow with piperine enhancement.
- **Remove piperine**: The bioavailability boost may concentrate fisetin in the liver too aggressively. Test 1,500mg without piperine vs. 1,000mg with piperine to separate dose from exposure.
- **Consider enteric-coated capsules**: Bypass the liver's first-pass metabolism.

Scenario 8: No Difference at All

⚠️ **What If This Happens?**

Problem: All three arms look identical. No biomarker moves in any direction.

🔧 **Adjustment**

- **Verify supplements reached the bloodstream**: LC-MS/MS for fisetin, NMN metabolites, spermidine in blood 2h post-dose (pharmacokinetic sub-study on n=5 per arm).
- **Check adherence**: Electronic pill bottles or capsule counting.
- **Consider that senescent cells may be resistant to natural senolytics**: In mice, fisetin works. In humans, the SCAP network may be more robust. Consider dasatinib-based approaches.
- **Accept negative result**: Publish. The community learns from well-designed negative studies. Not every hypothesis survives contact with reality.

Scenario 9: Only NMN/Spermidine Work, Fisetin Doesn't

⚠️ **What If This Happens?**

Problem: NAD+ increases, autophagy markers improve, IL-6 decreases — but tissue p16 is unchanged. Fisetin's senolytic effect failed while the metabolic support worked.

🔧 **Adjustment**

- **Report as "metabolic improvement without senescent cell clearance"**: This is still a valuable finding. It separates the two mechanisms.
- **Replace fisetin in V3**: Try dasatinib + quercetin (proven in humans) or navitoclax (if safety allows). Keep NMN + spermidine.
- **Or try higher fisetin with different delivery**: Liposomal fisetin (IV or oral) may achieve 10× higher tissue concentrations.

Decision Tree: Day 30 Checkpoint

```
Day 30 Results (Blood + Liver Panel)
│
├── Liver OK (ALT/AST normal)?
│   ├── YES → Continue all arms.
│   └── NO (>3× ULN in high-dose arm)
│       └── STOP 1,000mg arm. Safety Monitor review. Continue 500mg + placebo.
│
├── NAD+ increased?
│   ├── YES → NMN working. Continue.
│   └── NO → Increase to 500mg. Check absorption. Consider NR switch.
│
├── IL-6 changed?
│   ├── DECREASED → Good. SASP clearance working.
│   ├── UNCHANGED → Normal early. Recheck at Day 60.
│   └── INCREASED + liver OK → Clearance spike. Document kinetics.
│
├── Dose-response visible (500mg vs 1,000mg)?
│   ├── YES → Perfect. The 3-arm design is paying off.
│   └── NO → Both doses may be too low, OR fisetin doesn't work orally.
│       → Plan tissue fisetin measurement (LC-MS/MS on biopsy).
│
└── Overall:
    ├── Signals trending right → Continue. Await tissue data at Day 120.
    ├── Mixed → Continue. The tissue biopsy will clarify.
    └── All flat → Check adherence + pharmacokinetics before concluding.
```

Troubleshooting Matrix (V2)

Problem	Most Likely Cause	First Adjustment	Escalation
Tissue p16 unchanged	Fisetin doesn't reach dermis	Measure tissue fisetin (LC-MS/MS)	Add topical fisetin in V3
Dose-response but not significant	Underpowered (n=15)	Report trend + power calc for V3	Phase 2 with n=45/arm
IL-6 increased	Clearance spike or hepatotoxicity	Check liver panel	Add anti-inflammatory bridge
NAD+ unchanged	Poor absorption / CD38	Increase dose, sublingual	Switch to NR + apigenin
SDF1 unchanged (tissue)	Slow fibroblast turnover	Extend biopsy to Month 6-9	Drop as primary endpoint
Spots unchanged (but biomarkers improved)	Dermal melanin slow	Trust Masson-Fontana data	Extend to 9-12 months
Liver toxicity (high-dose arm)	1,000mg + piperine too aggressive	Stop high-dose arm	Test lower dose without piperine
No difference at all	Supplements not absorbed / senolytic resistance	Pharmacokinetic sub-study	Try D+Q or different delivery

Golden Rules

1. Change ONE variable at a time
2. Tissue data > blood data > photos (trust the hierarchy)
3. Safety > data — always
4. A dose-response trend is a success even without individual significance
5. Negative results are still results. Publish them.
6. Talk to your participants — they notice things tests can't

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"Science is not about being right. It's about finding out what's true."